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Exploiting *Xylella fastidiosa* proteins for Pierce's disease control

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Introduction

Xylella fastidiosa (Xf) cells, whether freshly grown or boiled, induce a chlorosis when pressure infiltrated into *Chenopodium quinoa* leaves

The chlorosis-inducing activity is associated with the presence of translation elongation factor "temperature-unstable" (EF-Tu)

EF-Tu is highly conserved among eubacteria and is an abundant protein

EF-Tu is among the proteins that is recognized by certain plants as a signal of the presence of bacteria (EF-Tu is a PAMP = pathogenesis-associated molecular pattern)

In some bacteria, EF-Tu has functions in addition to participating in protein synthesis. **An isoform of the EF-Tu of *Lactobacillus johnsonii* accumulates on the bacterial cell surface and mediates attachment of the bacterium to human intestinal cells and mucins** (Granato et al. 2004 , Infection and Immunity 72: 2160-2169)

We have found that the bulk of Xf EF-Tu behaves very unlike *E. coli* EF-Tu, which can be extracted as a typical soluble protein

Sequence comparison, EF-Tu from Xf and from *E. coli*

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Ec EF-Tu   Ac-SKEKFERTKPHVNVGTIGHVDHGKTTLTAAITTVLAKTYGGAARAFDQIDNAPEEKARG  60
Xf EF-Tu   Ac-AQDKFKRTKLHVNVGTIGHVDHGKTTLTAALTKVGAERFGGEFKAYDAIDAAPEEKARG  60

Ec EF-Tu     ITINTSHVEYDTPTRHYAHVDCPGHADYVKNMITGAAQMDGAILVVAATDGPMPQTREHI  120
Xf EF-Tu     ITISTAHVEYETEVRHYAHVDCPGHADYVKNMITGAAQMDGAILVCSAADGPMPQTREHI  120

Ec EF-Tu     LLGRQVGVPYIIVFLNKCDMVDDEELLELVEMEVRELLSQYDFPGDDTPIVRGSALKALE  180
Xf EF-Tu     LLARQVGVPYIVVFLNKADMVDDAELLELVEMEVRELLSKYDFPGDDTPIVRGSALKALE  180

Ec EF-Tu     GDAE--WEAKILELAGFLDSYIPEPERAIDKPFLLPIEDVFSISGRGTVVTGRVERGIIK  238
Xf EF-Tu     GDQSEIGVPAIIRLAEALDTHIPNPERAIDRPFLMPVEDVFSISGRGTVVTGRVECGVIK  240

Ec EF-Tu     VGEEVEIVGIKETQKSTCTGVEMFRKLLDEGRAGENVGVLLRGIKREEIERGQVLAKPGT  298
Xf EF-Tu     VGDEVEIVGIRPTSKTIVTGVEMFRKLLDQGQAGDNAGLLLRGTKRDEVERGQVLAKPGS  300

Ec EF-Tu     IKPHTKFESEVYILSKDEGGRHTPFFKGYRPQFYFRTTDVTGTIELPEGVEMVMPGDNIK  358
Xf EF-Tu     IKAHKEFEAEVYVLSKEEGGRHTPFFNGYTPQFYMRTTDITGKVCLPEGVEMVMPGDNVK  360

Ec EF-Tu     MVVTLIHPIAMDDGLRFAIREGGRTVGAGVVAKVLG  394
Xf EF-Tu     VTVSLINPVAMGEGQRFAIREGGRTVGAGVVSKVIG  396
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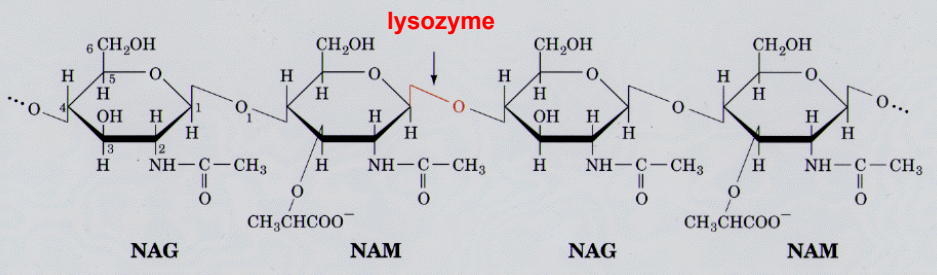
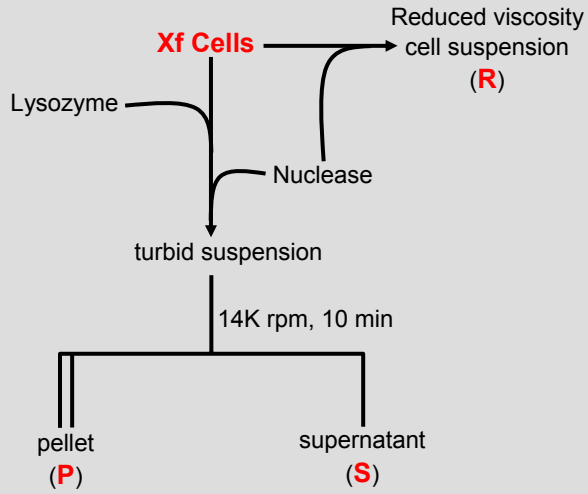
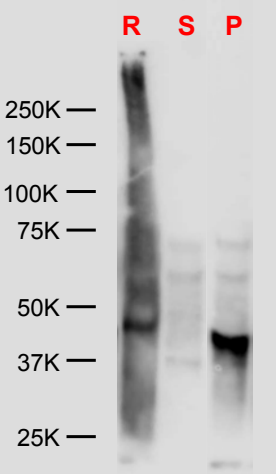
Red font shows sites at which Xf EF-Tu differs from *E. coli* EF-Tu

The sequence underlined in red was the immunogen for polyclonal antibody production



The bulk of the EF-Tu of Xf cells is in a form that is not solubilized by hot SDS treatment except after prior lyzozyme treatment





Immunoblot, anti Xf EF-Tu

Even after treatment with lysozyme, Xf EF-Tu remained particulate, though it could be solubilized in hot SDS solution; note that material high in lane R probably is under-represented because high molecular weight materials transfer poorly in immunoblots

EF-Tu purified from *E. coli* and a particulate fraction from lysozyme-treated Xf cells both induce chlorosis after pressure infiltration into *Chenopodium quinoa* leaves

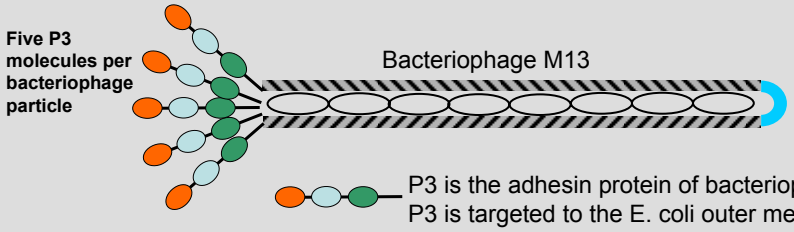
E. Coli EF-Tu was purified by covalent chromatography

Left side: filtrate from a 30K cut-off filter (i.e., buffer)

Right side: retentate from a 30K cut-off filter (i.e., retained EF-Tu protein in buffer)

Both sides of the leaf were infiltrated with a suspension corresponding to the 14K pellet (P fraction) from Xf cells treated with lysozyme

Targeting an EF-Tu-P3 fusion protein to the *E. coli* cell outer membrane

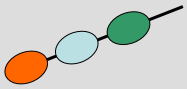


P3 is the adhesin protein of bacteriophage M13
P3 is targeted to the E. coli outer membrane

Two E. coli strains were prepared:

1) expressing a Xf EF-Tu-P3 fusion **2) expressing P3**

Xf EF-Tu-P3

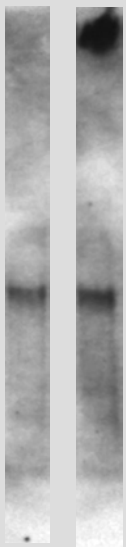


P3

Both P3 and EF-Tu-P3 are expected to be targeted to the outer membrane

When expressed in *E. coli*, a Xf EF-Tu-P3 fusion protein remained in an aggregated form after boiling cells in SDS solution

***E. Coli* strain expressing P3 protein**



Bottom of sample well

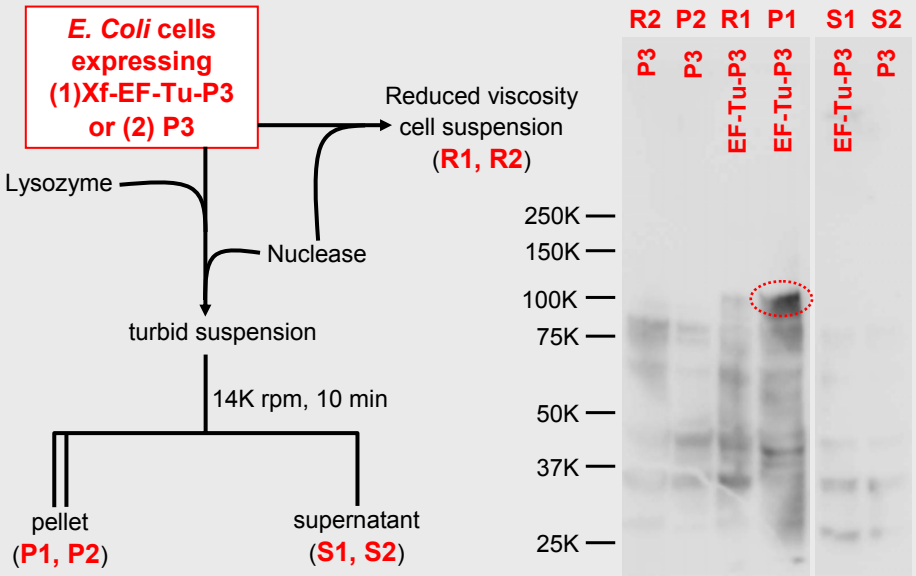
***E. Coli* strain expressing a Xf EF-Tu-P3 fusion protein**

E. coli EF-Tu

Immunoblot, detection with anti-*E. coli* EF-Tu

Heating in alkaline SDS-urea solution in the presence of mercaptans did not alter the result shown here

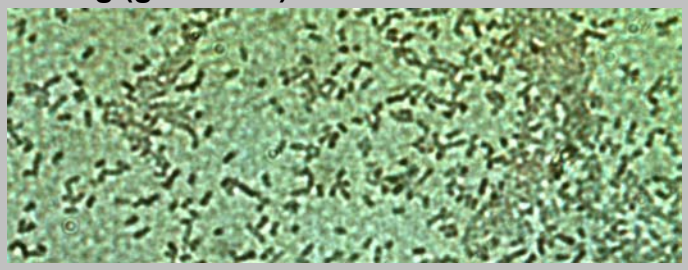
When expressed in *E. coli*, a Xf EF-Tu-P3 fusion protein is released in particulate form by lyzozyme treatment of the cells



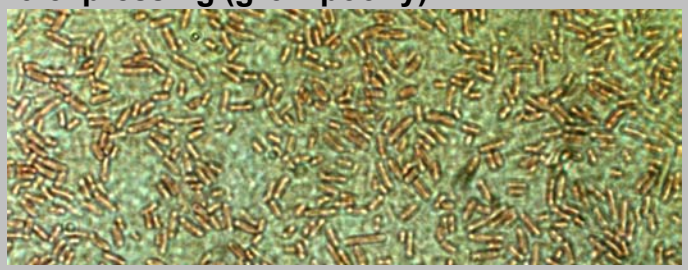
Even after treatment with lysozyme, Xf EF-Tu-P3 remained particulate, though SDS solubilizable

E. coli cells expressing EF-Tu-P3 were enlarged

P3-expressing (grow well)



EF-Tu-P3-expressing (grow poorly)



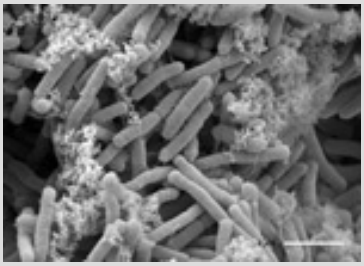
E. coli cells were stained with safranin

Results presented here are consistent with

(1) localization of an abundant isoform of Xf EF-Tu in the cell wall or outer membrane or both, perhaps covelently bound

(2) a role for Xf EF-Tu in determining cell shape: *E. coli* cells expressing an EF-Tu fusion protein were significantly elongated compared to control cells; Xf cells also are elongated

Future work will be aimed at determining whether Xf EF-Tu is exposed on the cell surface and, if so, what function it may perform in the Xf infection cycle



Xf cells